De novo Design and Engineering of Soluble Artificial Kinase Receptor Proteins

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Introduction

A major class of proteins associated with cancer are receptor tyrosine kinases (RTKs), which are involved in molecular signaling related to cell growth and proliferation. RTKs are composed of an integral membrane protein domain that is embedded in the membrane and a cytosolic (soluble) kinase domain. The cytosolic kinase domain becomes activated upon dimerization mediated by the integral membrane domain upon binding to biosignaling molecules (hormones). Drug therapies targeting the RTKs have largely focused on designing molecules that interact with the integral membrane domain, which binds to the signaling molecules, but have largely overlooked the kinase domain. Developing drugs to the kinase domain is difficult since the RTK protein is insoluble due to the presence of the integral membrane portion and removal of the protein from the membrane for studying drug binding can lead to denaturation and inactivation of the protein. The research presented here looks at developing artificial kinase receptors by replacing the integral membrane domain with soluble coiled coil forming peptides that can mimic the normal function of the integral membrane domain, i.e. dimerization, but produce a soluble artificial kinase receptor fusion protein mimic that can be used in drug screening assays to discover new RTK kinase domain inhibitors.

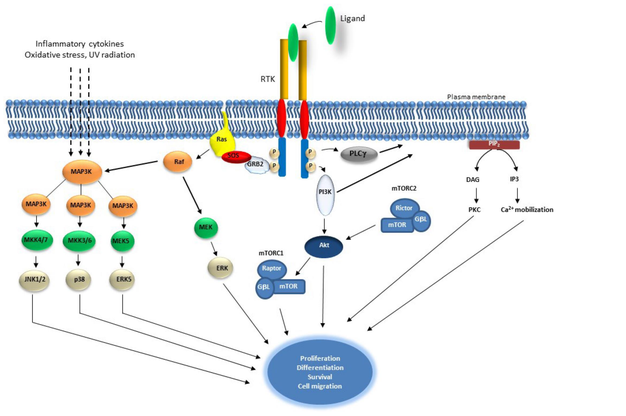


Figure 1. Schematic representation of receptor tyrosine kinase and downstream signaling pathways.[1]

Receptor Tyrosine Kinase Structure and Dimerization

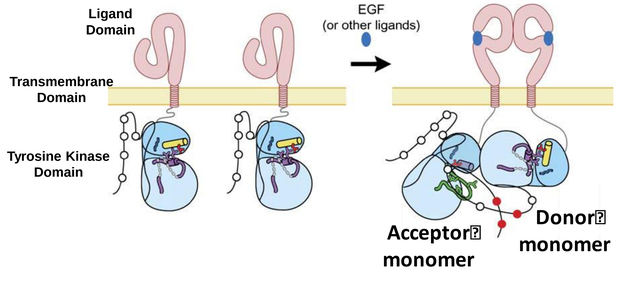


Figure 2. Asymmetric dimer formation and activation of kinase domains (adapted from reference 2).

-RTKs are insoluble membrane bound proteins due to the integral transmembrane domain.

-Dimerization is essential from activation of the kinase domains upon binding of a ligand.

-Drugs for RTKs largely target the ligand binding/transmembrane domain.

Developing Soluble RTK Mimics for Kinase Domain Drug Discovery

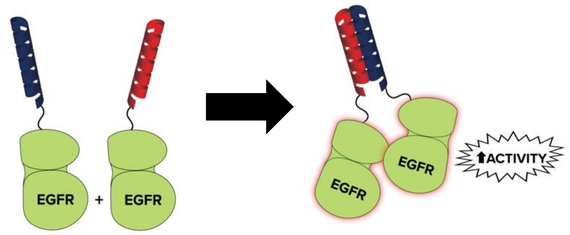
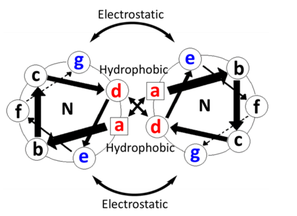


Figure 3. Design of a soluble RTK protein mimic comprised of the EGFR kinase domain.

-Developing drugs targeting the kinase domain (KD) is difficult due to the presence of the ligand/transmembrane domains and insoluble nature of the transmembrane domain.

-Developing a soluble protein that contains the KD (EGFR in figure 3), but has a soluble domain that mediates dimerization would be useful for screening KD drug candidates.

Coiled Coil Peptides as Soluble Transmembrane Domain Mimics



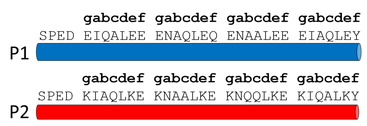


Figure 5. Heterodimeric parallel coiled coil peptide design.[3]

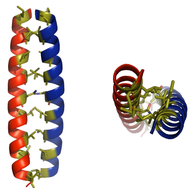


Figure 4. Coiled coil helical wheel (top) and cartoon model (bottom) .

-Coiled coils are structural motifs found in proteins whereby 2 or more alpha-helices wrap around one another.

-Coiled coils are characterized by a heptad repeat of amino acids (**abcdefg**) where the **a**/**d** positions are hydrophobic and **e**/**g** positions are charged ionic amino acids.

-*De novo* coiled coil design is well developed to control both oligomeric state, parallel/antiparallel orientation and specificity of interactions.

Molecular Construction of "Soluble RTK" Proteins

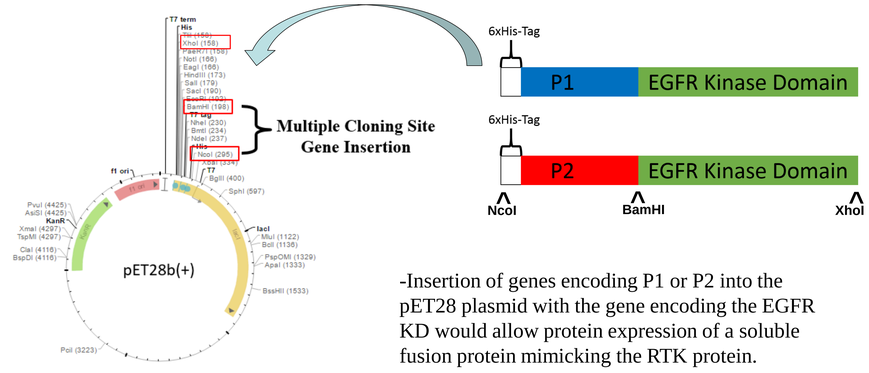


Figure 6. pET28b vector indicating NcoI, BamHI , and XhoI cloning sites for gene insertion.

-Insertion of genes encoding P1 or P2 into the pET28 plasmid with the gene encoding the EGFR KD would allow protein expression of a soluble fusion protein mimicking the RTK protein.

Preliminary Protein Expression and Purification of P1-EGFR

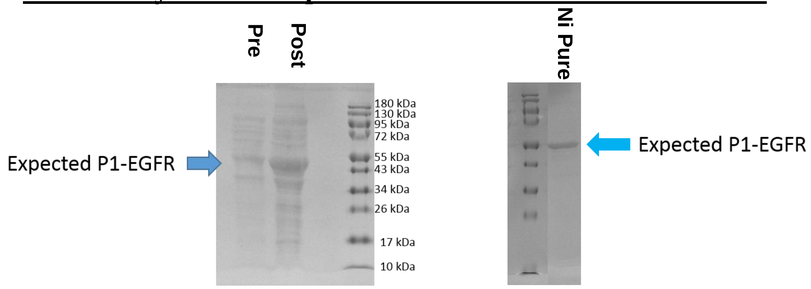


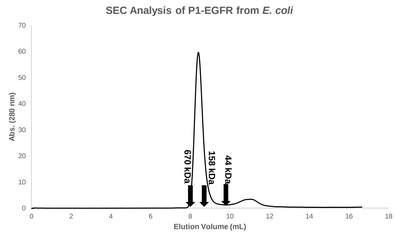
Figure 8. Expression and purification results for P1-EGFR. Left) Preliminary protein expression studies of P1-EGFR KD (expected molecular weight 44.7 kDa). Right) P1-EGFR purified by denatured nickel (Ni) affinity chromatography produced highly pure soluble protein.

-Preliminary expression performed overnight at 30 °C showed a strong band by SDS-PAGE corresponding well with the molecular weight of P1-EGFR (44.7 kDa expected).

-Purification by nickel affinity yielded a soluble peak, but had a very low yield.

-Denatured nickel affinity chromatography produced better yields of soluble pure protein.

Analysis of P1-EGFR



-Size exclusion chromatography of refolded P1-EGFR showed large molecular weight aggregation.

-E. coli expression is known to yield misfolded and inactive protein.

-Expression of P1-EGFR in insect cells has produced soluble, active protein that shows self dimerization of P1.

Conclusions and Future Directions

-E. coli expression of P1-EGFR produces insoluble protein that cannot be refolded into an active conformation.

-Expression of P1-EGFR in insect cells has shown active soluble protein due to self dimerization of the P1 that was not reported in the literature.

-On-going efforts are focused on insect cell expression using an alternate set of coiled coil peptides that have been used in protein dimerization.

Acknowledgements

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